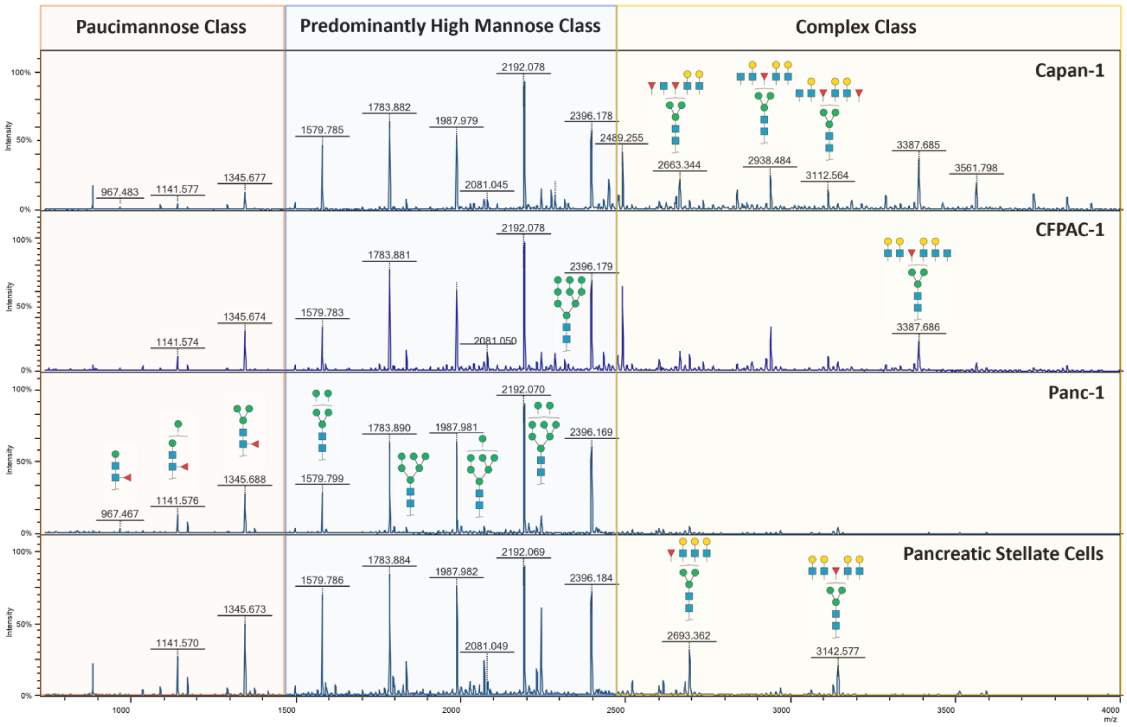
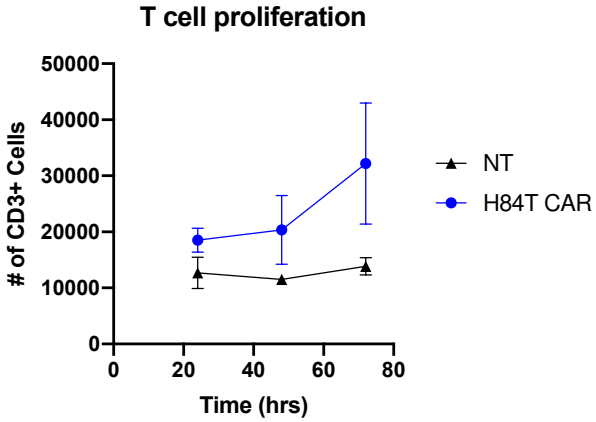
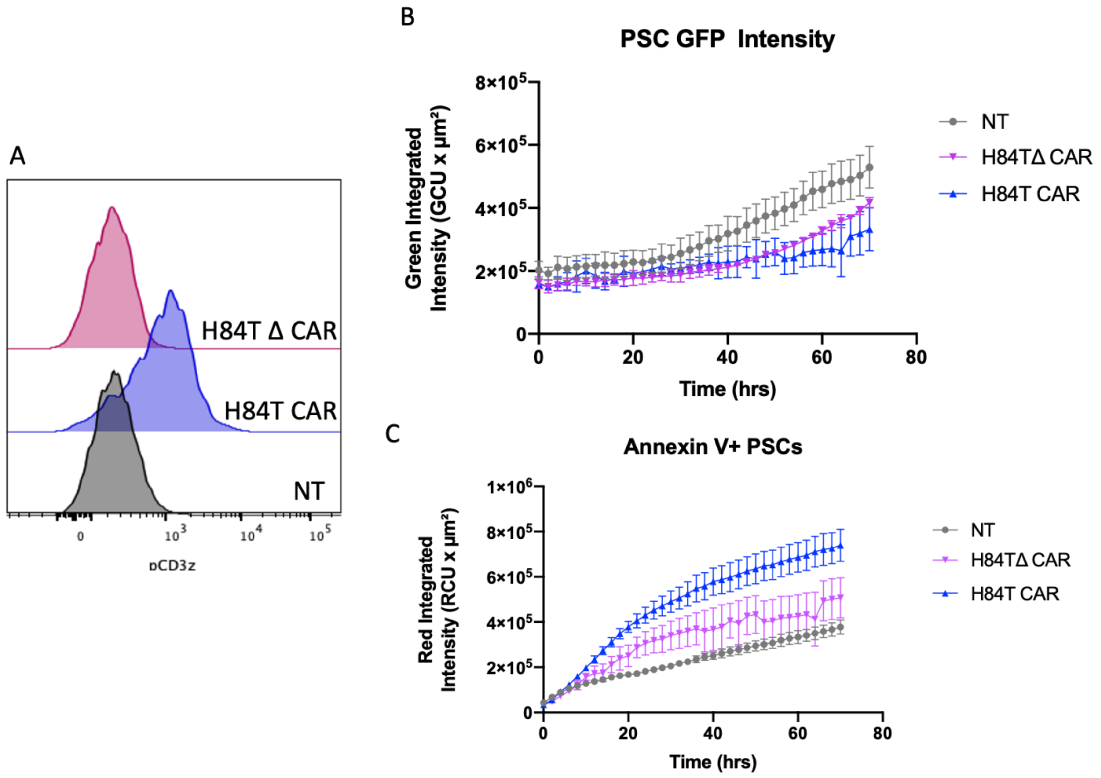
Supplemental Figures



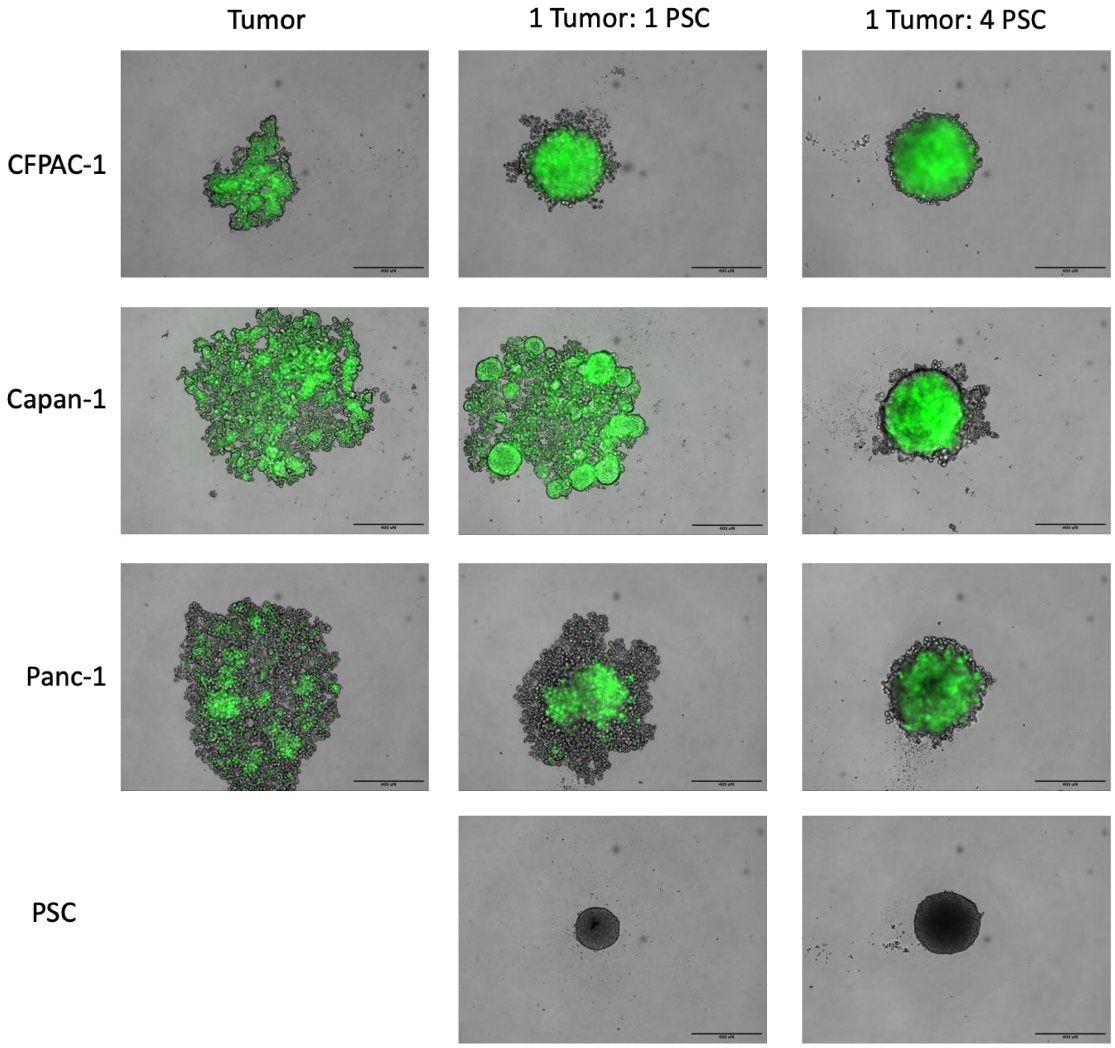
Suppl. Figure 1: PDAC and PSC glycan profiles. Intensity of particular glycans structures shown were quantified by MALDI-TOF mass spectrometry.



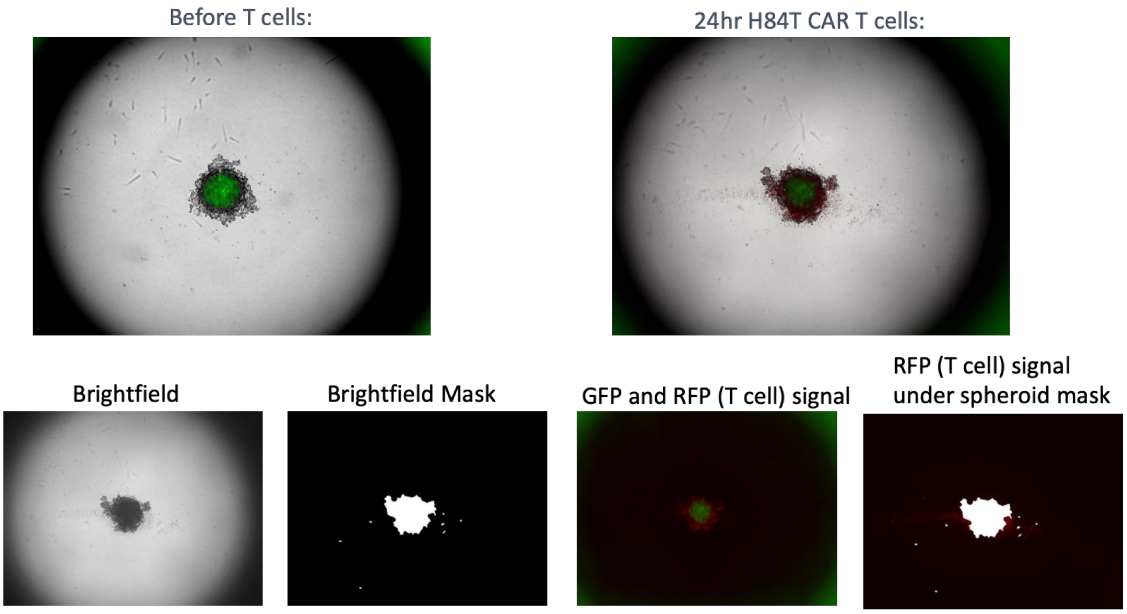
Suppl. Figure 2: H84T CAR T cell expansion. $4x10^4$ CFPAC-1 tumor cell lines and $1x10^4$ NT or H84T CAR T cells were co-cultured. T cells were quantified by CD3+ staining and counting beads collected by flow cytometry.



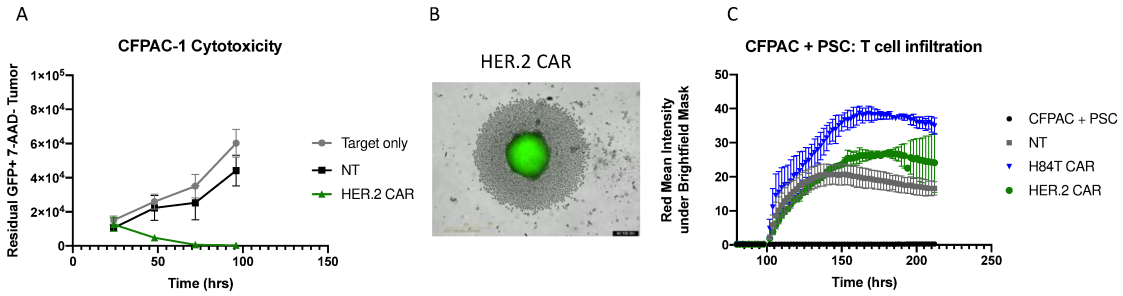
Suppl. Figure 3: H84T signaling deficient CAR T cells. A) Non-transduced, H84T and H84TΔ CAR T cells were fixed and stained for phospho-CD3ζ (CD247) and analyzed by flow cytometry. B) $8x10^3$ PSCs were seeded in 96-well 1% agarose coated plates. $2x10^3$ T cells were added and green integrated intensity of CSFE labeled PSC spheroids were quantified by Incucyte live image analysis post T cell addition over time. Results are an average of 4 technical replicates of 3 different donors. B) Annexin V staining quantification of PSC spheroids treated with indicated T cells by Incucyte live image analysis.



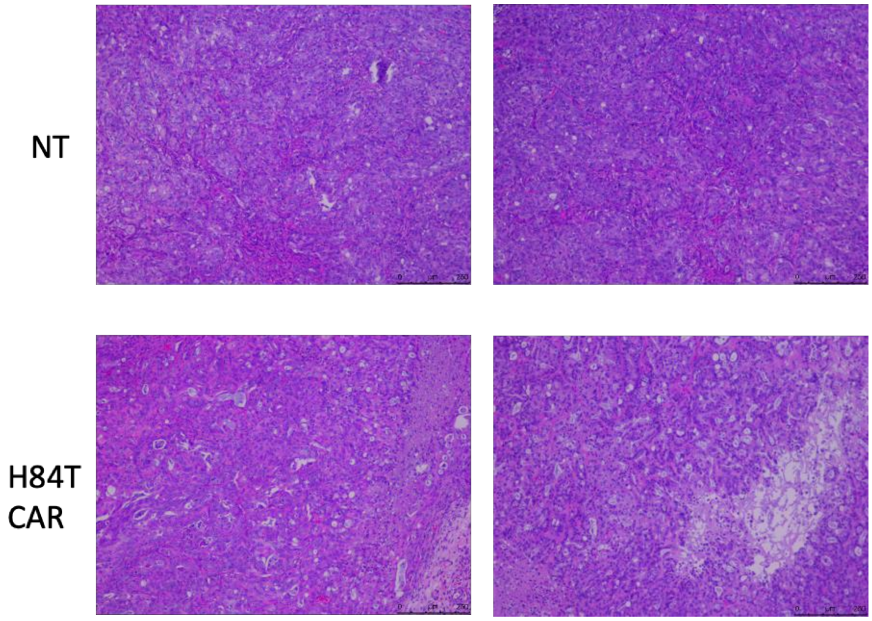
Suppl. Figure 4: PDAC tumor cell lines form 3D spheroids with the addition of PSCs. $2x10^3$ GFP labeled tumor cell lines were added to 1% agarose coated 96 well plates. $2x10^3$ or $8x10^3$ PSCs were added to tumor cell lines and spheroids formed. Images were acquired 48hr post tumor and PSC cell addition by Incucyte live image analysis.



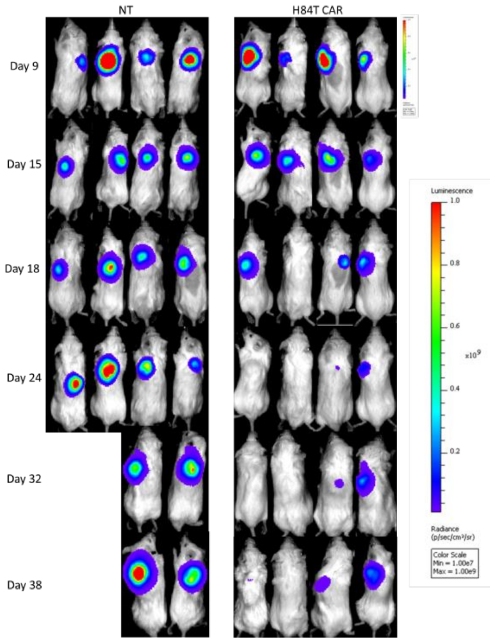
Suppl. Figure 5: T cell infiltration assay on Incucyte live imaging system. $2x10^3$ GFP labeled CFPAC-1 tumor cells and $8x10^3$ PSCs were added to 1% agarose coated 96 well plates. $1x10^3$ cell tracker red labeled T cells were then added 96hrs later. Whole spheroid analysis was performed. The brightfield mask was used to define the borders to detect the RFP signal from T cells. RFP intensity was calculated by Incucyte imaging software and measured over time as shown in Figure 5.



Suppl Figure 6: HER.2 CAR T cells target PDAC tumors but cannot infiltrate PSC spheroids. A)Co-culture of CFPAC-1 PDAC tumor cell lines with NT or HER.2 CAR T cells at a 4:1 ratio. Residual viable tumor cells labeled with GFP fFluc were quantified by flow cytometry, 7-AAD staining and absolute counting beads. Samples were collected at 24, 48, 72, and 96hrs post T cell addition. n=4 donors. B) $4x10^3$ PSCs labeled with CSFE were seeded in a 1% agarose coated 96-well plate and allowed to form spheroids for 24hrs. $2x10^3$ NT or HER.2 CAR T cells were then added and images were acquired every 2hrs for 4 days by Incucyte live imaging system. Representative image of one donor T cell and spheroids are shown 96hr post T cell addition. C) $2x10^3$ GFP labeled CFPAC-1 tumor cells and $8x10^3$ PSCs were co-cultured in 1% agarose coated 96 well plates for 96hrs. $1x10^3$ NT, H84T or HER.2 CAR T cells labeled with cell tracker red were then added and spheroids were imaged every 2hrs on the whole spheroid imaging setting. The red intensity of T cells quantified under the brightfield mask is graphed. Average of 2 donors with quadruplicates are shown. Linear regression analysis comparing the slope of H84T CAR vs HER.2 CAR $p<0.001$.



Suppl. Figure 7: Representative H&E staining of resected tumors treated with either NT or H84T CAR T cells. Images were then converted to binary signal with ImageJ analysis software and pixel count was quantified to determine tumor density.



Suppl. Figure 8: NSG MHC KO mice were engrafted subcutaneously with $3x10^6$ GFP-FfLuc labeled Capan-1 tumor cells and $3x10^6$ PSCs. Tumors were allowed to establish for 1 week and then $1x10^6$ NT or H84T CAR-T cells were delivered IV. Tumor signal was quantified by bioluminescence signaling through IVIS imaging and images for Figure 6 are shown here. N=4 mice per group.